## Module Overview

<table>
<thead>
<tr>
<th>Day</th>
<th>Lecture</th>
<th>Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction</td>
<td>DNA library synthesis (PCR)</td>
</tr>
<tr>
<td>2</td>
<td>SELEX I: Building a Library</td>
<td>DNA library purification (agarose gel electrophoresis)</td>
</tr>
<tr>
<td>3</td>
<td>SELEX II: Selecting RNA with target functionality</td>
<td>RNA library synthesis (<strong>In vitro</strong> transcription = IVT)</td>
</tr>
<tr>
<td>4</td>
<td>SELEX III: Technical advances &amp; problem-solving</td>
<td>RNA purification and heme affinity selection</td>
</tr>
<tr>
<td>5</td>
<td>Characterizing aptamers</td>
<td>RNA to DNA by RT-PCR</td>
</tr>
<tr>
<td>6</td>
<td>Introduction to porphyrins: chemistry &amp; biology</td>
<td>Post-selection IVT <strong>Journal Club 1</strong></td>
</tr>
<tr>
<td>7</td>
<td>Aptamer applications in biology &amp; technology</td>
<td>Aptamer binding assay</td>
</tr>
<tr>
<td>8</td>
<td>Aptamers as therapeutics</td>
<td><strong>Journal Club 2</strong></td>
</tr>
</tbody>
</table>
Therapeutic Aptamers

20.109 Lecture 8
3 March, 2011
Today’s Objectives

• Aptamers developed for therapeutic purposes:
  – Focus on one disease process
  – Gain an appreciation for:
    • Defining the problem you are addressing on several different levels:
      – Organism
      – Anatomical
      – Molecular
    • Developing solutions based on understanding disease mechanism
  • Some challenges in translating your molecular level solutions (aptamer) into efficacious drugs (in people)
Age-related macular degeneration (AMD)

• Disease affecting the eye
  – Most common cause of *irreversible* vision loss in the developed world

  – 8 million in the U.S. are affected

  – Typically, incomplete vision loss
    • Non-life threatening
    • Enough to impair independence in daily living activities
Anatomy of the eye

- Sclera
- Choroid
- Retina
- Cornea
- Pupil
- Lens
- Iris
- Ciliary body
- Fovea
- Optic nerve
- Vitreous humor

Gross Anatomy of the Eye, 2007 Update (NCBI Bookshelf)
Anatomy of the eye

• **Retina**
  – Light sensitive part of the eye

• **Macula**
  – Highest density of light sensitive receptors in this region
    • Highest visual acuity

• **Fovea**
  – Cones concentrated here
  – Most light entering the eye is focused here
    • Color vision
    • High acuity vision

Observed retinal changes during AMD

- Yellow-white deposits (drusen) appear in the macula
- Enlarged as the disease progresses
- New-blood vessels may develop (neovascularization)
  - Retinal hemorrhages may occur

Consequences of these retinal changes

• Macula function disrupted
  – Region of highest visual acuity is damaged
  – Central vision can be severely affected

Age-related macular degeneration (AMD)

- **Risk factors**
  - Age (primary risk factor)
    - “Middle aged” -- 2% risk
    - ≥ 75 years -- 30% risk
  - Smoking
  - Obesity
  - Race (highest in caucasians)
  - Family history

- **Two sub-types of AMD**
  - **Dry AMD**
    - No neovascularization
    - Large drusen deposits
  - **Wet AMD**
    - Neovascularization present
    - Sub-retinal hemorrhages possible
    - ~10-15% of all AMD cases
    - Responsible for ≥ 80% severe vision loss due to AMD!
Disease pathophysiology

• Not fully understood

• **Wet AMD:**
  – High Vascular Endothelial cell Growth Factor (VEGF) levels present in the eye
• Impacts endothelial cell function
  – Endothelial cell = special cell type lining the interior of all blood vessels
  – Endothelial cells in all blood vessels respond to VEGF

• VEGF affects endothelial cell:
  – Proliferation
  – Differentiation
  – Permeability

http://www.researchvegf.com/researchvegf/index.m (Genentech)
Disease pathophysiology

- Not fully understood

- **Wet AMD:**
  - High Vascular Endothelial cell Growth Factor (VEGF) levels present in the eye
  
  - VEGF is *pro-angiogenic* (promotes new blood vessel growth)
    - New blood vessels are more fragile
    - Leakiness/rupture leads to hemorrhage & vision loss

  - **What are some possible approaches to treating wet AMD based on this information?**
Treatment options for Wet AMD

- **Photodynamic therapy**
  - Aimed at directly treating new blood vessel formation
  - Photosensitizer drug injected systemically (entire body exposed)
  - Local irradiation of macula with red light
    - Verteporphin photo-activated in the presence of light and O\textsubscript{2} will produce reactive oxygen species (ROS)
    - ROS are toxic to nearby endothelial cells
Treatment options for Wet AMD

• **Photo-coagulation therapy**
  
    - Laser used to target new blood vessels growing in the macula
  
    - Does not prevent/slow disease progression
  
    - **Risks:**
      
      • Irreversible damage to surrounding retina
      
      • Further deterioration of visual acuity
Treatment options for Wet AMD

- **Anti-VEGF therapy**

  **Hypothesis:**

  - Elevated ocular VEGF levels are directly responsible (at least in part) to increased new blood vessel formation

  - Inhibiting VEGF activity can significantly reduce new blood vessel growth

    - Slows rate of vision loss by reducing retinal hemorrhages

    - Note: Treatment impacts Wet AMD only!
Treatment options for Wet AMD

- **Anti-VEGF therapy**
  - First FDA approved anti-VEGF drug to treat wet AMD: Pegaptanib, sodium (Macugen)
  - Approved: 2004
Developing aptamers as therapeutics

- What are some significant challenges to overcome in developing RNA aptamers as therapeutic agents?
  - **Stability**
    - Nucleases
    - Chemical (e.g. metal catalyzed)
  - **Clearance**
    - Drug must accumulate to a therapeutic level
    - But not achieve a toxic level
    - Minimize dosing frequency
  - **Delivery method/bioavailability**
    - Oral, intravenous, etc.
Nuclease activity spectrum

- Nucleases can be categorized broadly as:

  - **Endonucleases**
    - Cleave internal phosphodiester bonds anywhere within a nucleic acid polymer

  - **Exonucleases**
    - Cleave phosphodiester bond
    - But only at or near a free terminus
    - Two types:
      - 5’-exonuclease
      - 3’-exonuclease
Anti-VEGF aptamer modifications reducing nuclease susceptibility

5′-terminus

NDA 21-756

3′-terminus
Nucleotide sugar modifications

- RNA is significantly stabilized by introducing 2'-sugar modified nucleotides:
  - Fluoro group
  - Methoxy group

- Impart stabilization against:
  - Endonucleases
  - Spontaneous cleavage
  - Metal-catalyzed (chemical) cleavage

- What mechanism(s) can you propose to explain this?
Sugar modified nucleotides and stability

**Spontaneous cleavage**

- 2’-OH group has sufficient nucleophilicity to initiate intramolecular reaction that leads to phosphodiester bond cleavage

- Fluorine is highly electronegative
  - Poor nucleophile
  - F does not attack the phosphate group
  - Cleavage reduced

Sugar modified nucleotides and stability

Metal ion-dependent cleavage chemistry

- Some metals can help deprotonate the 2'-OH group in normal RNA
  - Accelerate cleavage reaction
- Both the 2’-F and 2’-OMe derivatives prevent this chemistry
  - Impart stability to RNA

Same factors contribute to imparting resistance to RNases

Modified backbone linkages

Anti-VEGF RNA Aptamer!
Modified backbone linkages

- Typical linkage in DNA or RNA
  - 3’-5’ phosphodiester linkage

- Notice the presence of new linkage at the 3’-end of the aptamer
  - 3’-3’ phosphodiester linkage

- Provides significant resistance against 3’-exonucleases
  - Major nuclease activity present in serum
Modified backbone linkages

Anti-VEGF RNA
Aptamer!
Modified 5’-terminus

- What does this accomplish?
- Reduced susceptibility to 5’-exonucleases
- Reduced clearance
  - Increased size of PEG-conjugated aptamer
  - Pegaptanib, sodium molecular weight ~ 50 KDa
  - Aptamer alone: ~ 10 KDa

and n is approximately 450.
Why not eliminate all sites from which aptamer degradation may occur?

- Elimination may be undesirable: Aptamer function compromised
- Intentional: Modulates aptamer’s lifetime within patient (pharmacokinetics)
How do you deliver aptamer drugs to the retina

- **Anti-VEGF therapy**
  - Cannot be delivered systemically (e.g. intravenously)
    - Pro-thrombotic
  - Poor oral bioavailability
  - *Injected directly into the vitreous humor of the eye!*

Summary

• Aptamers have been successfully developed as therapeutics
  – Other aptamers in different stages of drug testing trials include:
    • Anti-clotting agents
    • Anti-cancer agents

• Several factors must be specifically addressed to achieve this:
  – Stability to nuclease-mediated degradation
  – Bioavailability
  – Pharmacokinetics
  – Delivery to target interaction site
  – Cost